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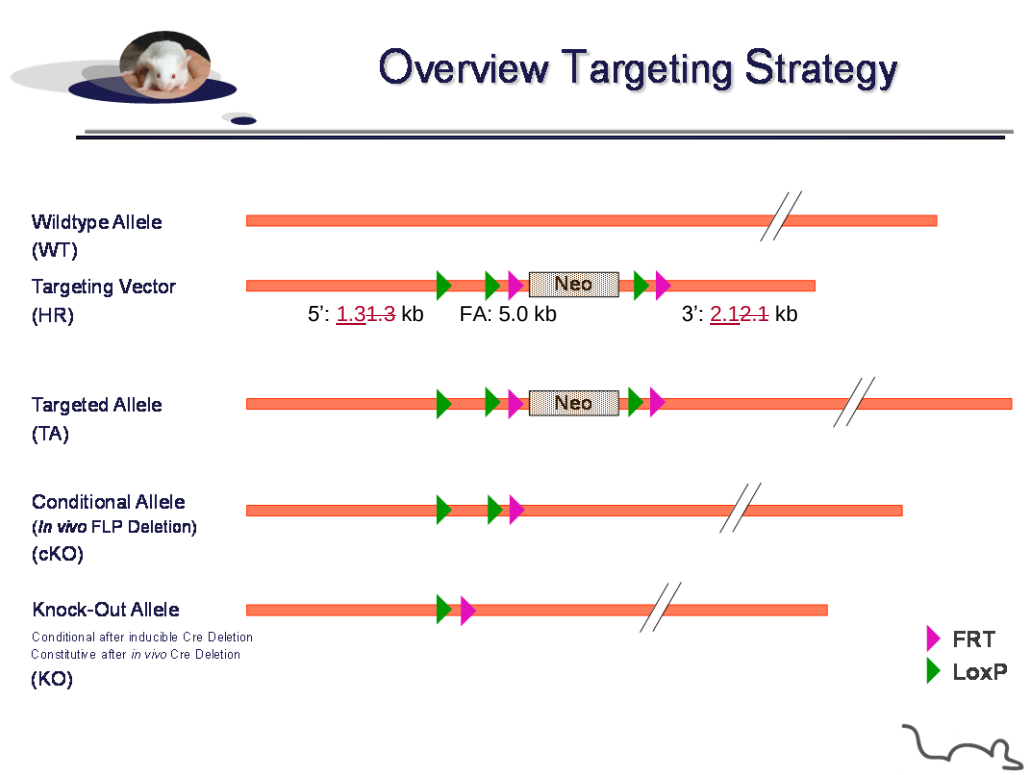
This protocol has been prepared by Claudia Caradec, Engineer
This protocol has been validated by Sylvie Jacquot, Ph.D., Project Manager

1. Schematic representation of the locus

1.1. Overview



Legend:
5': 5' homology arm; FA: floxed fragment; 3': 3' homology arm
This schematic representation is not on scale

1.2. Strategy chosen: flox of exons 4-5-6

Nr2c2 gene (also named TR4) is a member of the nuclear receptor family. Additional information on this gene can be accessed at:
http://www.informatics.jax.org/javawi2/servlet/WIFetch?page=markerDetail&key=45359

Strategy used to generate the conditional knock out model

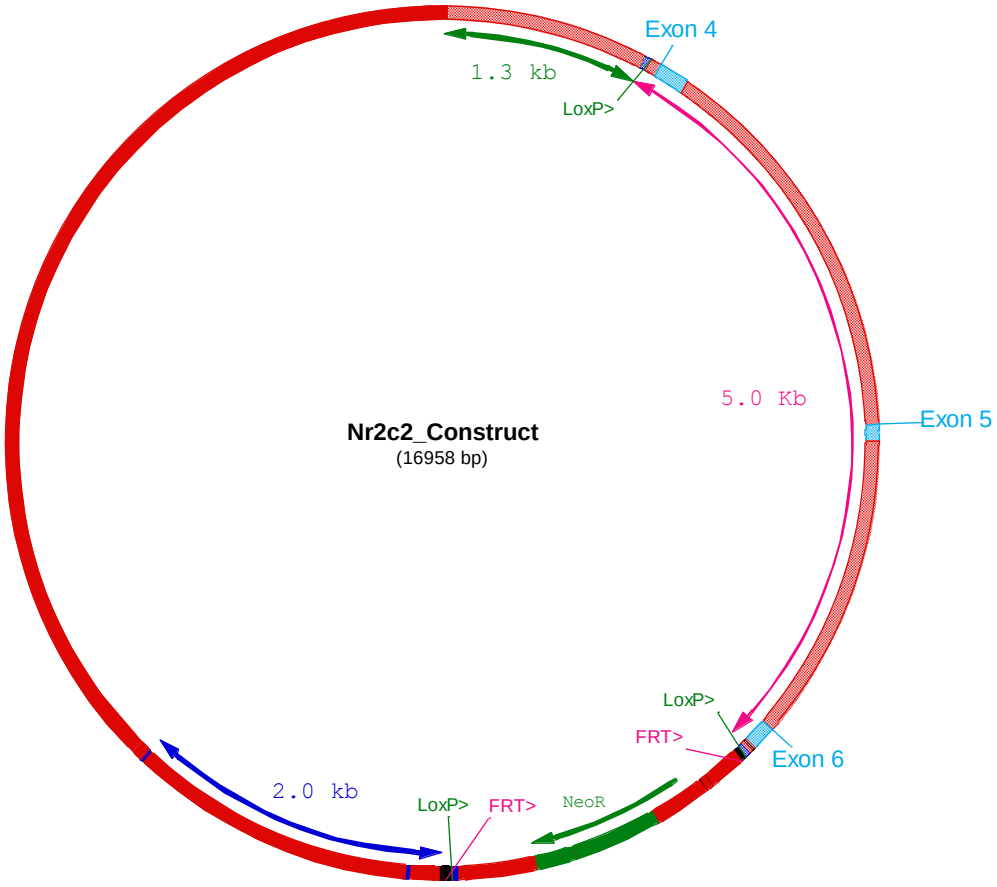
Nr2c2_Strategy
(10521 bp)

2. Construct used for homologous recombination in ES cells: Nr2c2 project

2.1. Legend

loxP sites are indicated in green ; FRT sites are indicated in purple; *Mus musculus* sequences are indicated in uppercase ; exogenous sequences are marked in lowercase.
The targeting vector was generated in 129Sv/Pas and was not fully sequenced.

2.2. Map of targeting vector plasmid



2.3. 5' homology arm (1.3 kb)

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2.4. Floxed fragment (5.0 kb)

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2.5. PGK-Neo region

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2.6. 3' homology arm (2.0 kb)

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2.7. Vector backbone sequence

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atggcggacgcgatggatatgttctgccaagtcagcgtttaaacttaattaagtcgacggcc

3. ES cell lines targeted and validation data:

3.1. ES cell lines targeted

The targeting vector was electroporated in P1 ES cells [MCI-129Sv/Pas background]

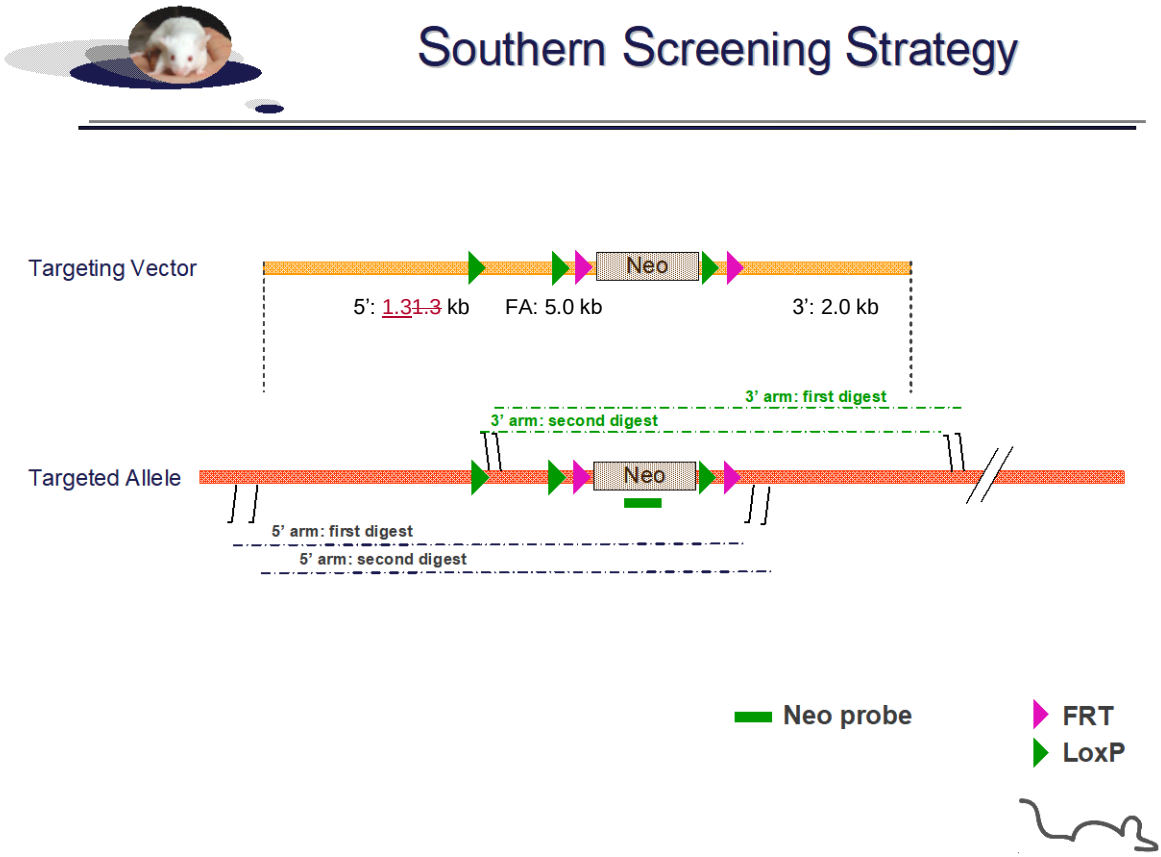
Number of clones screened: ~ 400
Number of positives: 1

Reference of clone used to generate the mouse line:

- clone **DG3-133**

3.2. Southern data on positive clone

3.2.1. Neo Southern strategy



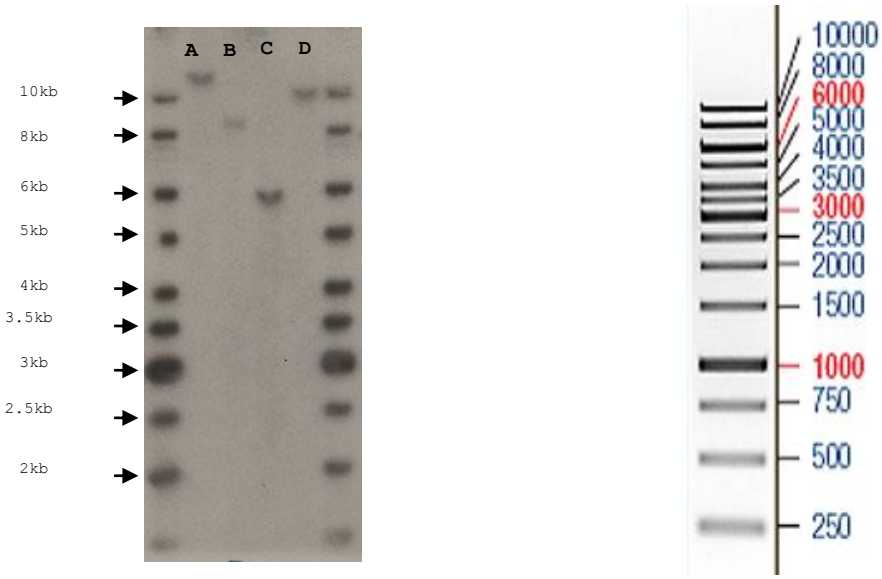
Digestions used to validate the 5' and 3' insertion

Probe	Name	Genomic DNA digest	WT allele (kb)	Targeted Allele (kb)
Neo	5' arm first digest	Afl II	/	11.5
	5' arm second digest	Xho I	/	8.6
	3' arm first digest	Ase I	/	5.6
	3' arm second digest	SspI	/	10.2

Four different digests are used to validate correct HR event. Two digests validate the 5' insertion, 2 other digests validate the 3' insertion

3.2.1. Picture of Neo Southern

Neo southern blot: 5’ and 3’ arm validation ladder



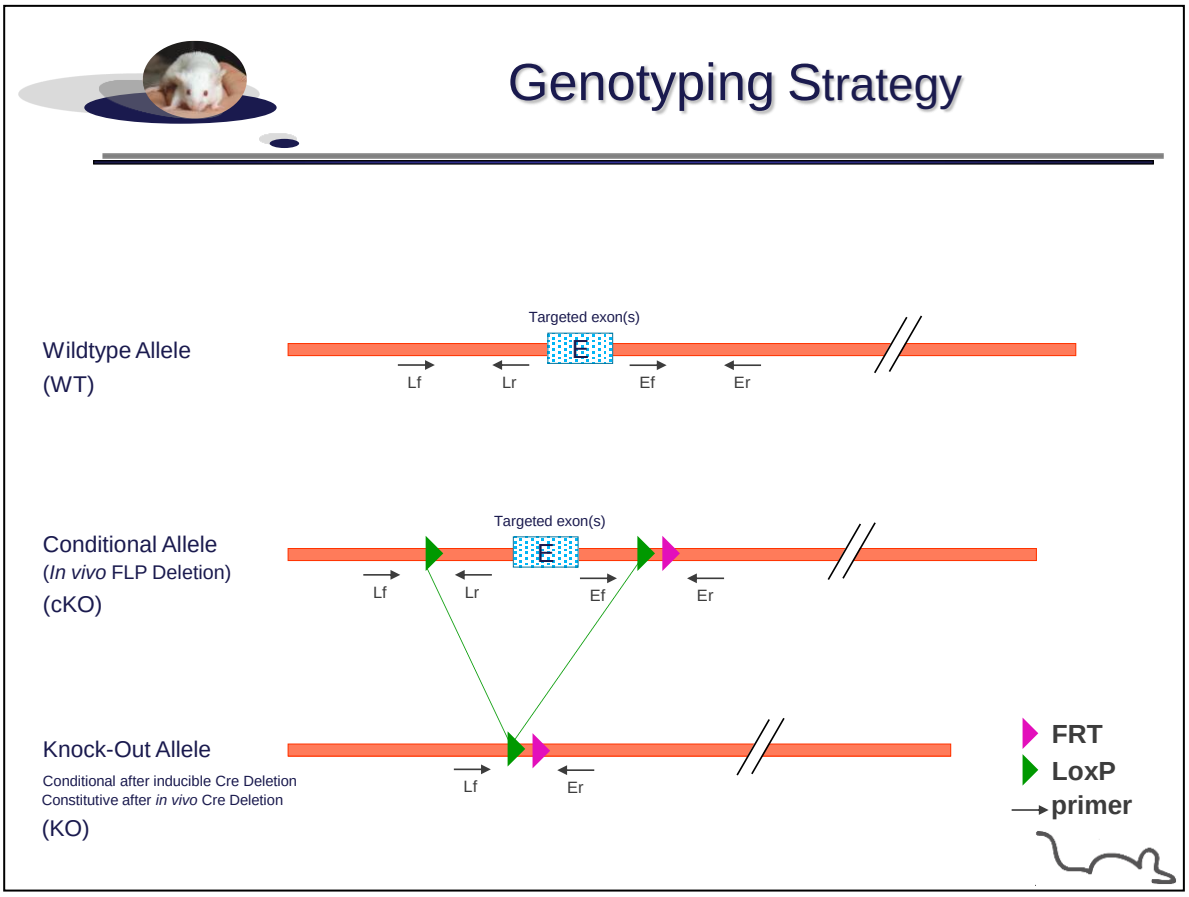
A: 5’ arm first digest (Afl II)
B: 5’ arm second digest (Xho I)
C: 3’ arm first digest (Ase I)
D: 3’ arm second digest (SspI)

4. Data on conditional and knock-out animals: Genotyping protocol and data:

Both conditional and knock-out mouse models were backcrossed in C57BL/6J background.

4.1. Genotyping strategy

The map below describes the position of the primers used for genotyping for each possible allele.



Sequence of primers used for genotyping

Position	Primers	Sequence
Lf	13	GTTTCCTTAGTGCTTCTAAATCCCG
Lr	14	CCCTTTGCTTGGGGTGTCTTTAGAC
Ef	308	GGAAGAATCTGACCTACAGCTGTCG
Er	410	CGTTACCTAATCTCCATTCCTCCTGTC

PCR fragments expected size (bp):

Region analyzed	**Primers used**	Position on the primer (see the map above)	Conditional allele (cKO)	Knock-Out allele (KO)	WT allele (wt)
Presence of the distal 5'loxP	13-14	Lf / Lr	217	---	163
Excision of the selection marker	308-410	Ef / Er	508	---	375
Total Excision (excision of the floxed exon(s), i.e. knock out)	13-410	Lf / Er	5348*	433	5161*

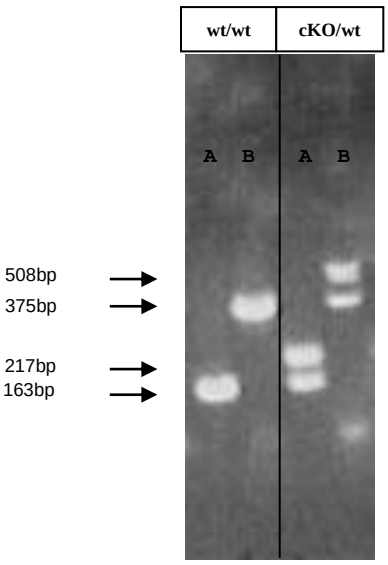
* This PCR product will not be observed using our PCR genotyping conditions (see description below)
--- No Amplicon should be obtained

4.2. Pictures of genotyping with various alleles

Analysis of PCR products pattern was done by gel electrophoresis (3% gel was used).

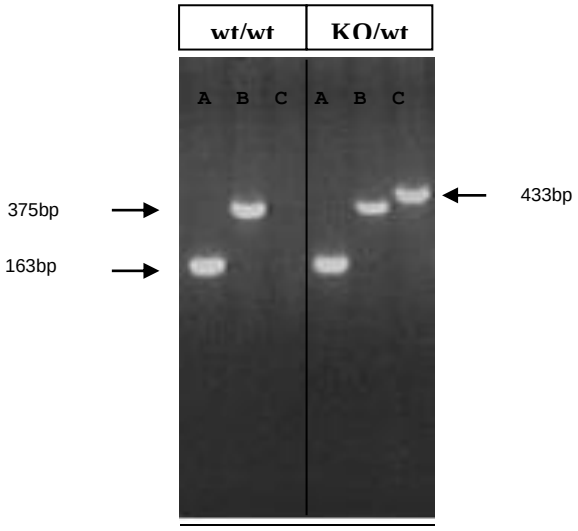
Representative genotyping picture for wt/wt and cKO/wt mice

A:Distal 5'LoxP
B:Excision of marker



Representative genotyping picture for wt/wt and KO/wt mice

A:Distal 5'LoxP
B:Excision of marker
C: Total Excision



Representative genotyping picture for wt/wt and KO/KO mice

A:Distal 5'LoxP
B:Excision of marker
C: Total Excision